



## Tracking chlordane compositional and chiral profiles in soil and vegetation

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### Abstract

The cycling of chlordane and other persistent organic pollutants through the environment must be comprehensively elucidated to assess adequately the human health risks posed from such contaminants. In this study the compositional and chiral profiles of weathered chlordane residues in the soil and vegetative compartments were investigated in order to provide details of the fate and transport of this persistent pesticide. Zucchini was planted in a greenhouse in three bays containing chlordane-contaminated soil. At harvest the vegetation and soil were extracted and analyzed for chlordane content using chiral gas chromatography/ion trap mass spectrometry. Both achiral and chiral chlordane components were quantified. The chlordane concentration in the rhizosphere (soil attached to roots) was significantly less than that in the bulk soil. However, the enantiomeric ratio of the chiral components and overall component ratios had changed little in the rhizosphere relative to the bulk soil. Significant levels of chlordane were detected in the vegetation, the amount varying in different plant tissues from a maximum in roots to a minimum in fruit. In addition to the chlordane concentration gradient in plant tissues, significant shifts in compositional profile, as indicated by the component ratios, and in chiral profile, as indicated by the enantiomeric ratio, of the contaminant were observed in the plant tissues. The data indicate that abiotic processes dominate the transport of the chlordane components through the soil to the plant. This is the first report of the effect of rapid biotic processes within the plant compartment on chlordane compositional and chiral profiles. © 2002 Elsevier Science Ltd. All rights reserved.

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### 1. Introduction

Persistent organic pollutants (POPs) are a class of compounds characterized by exceedingly long half-lives in the environment which are often on the order of years or decades (Wania and Mackay, 1996). Included in this group of highly persistent contaminants are DDT,

DDE, dieldrin, aldrin, toxaphene, and heptachlor. Also among the POPs, chlordane is an organochlorine insecticide, herbicide, and termiticide which was used extensively in the United States beginning in the 1940s. Due to its persistence in the environment (Nash and Woolson, 1967), the United States Environmental Protection Agency cancelled its registration in 1988 (US EPA, 1990). Although technical chlordane consists of over 140 different components, the three primary components in technical chlordane also persist in weathered residues:  $\alpha$ -(*cis*-)chlordane (CC);  $\gamma$ -(*trans*-)chlordane (TC); and *trans*-nonachlor (TN) (Mattina et al., 1999).

The total risk to human health posed from weathered chlordane and other POPs in the environment is

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uncertain. Certain POPs have been linked to endocrine disruption (McKinney and Waller, 1994) and mammalian carcinogenicity (Gosselin et al., 1984). The hydrophobic character of this class of contaminants and their long-term persistence indicate a high probability of bioaccumulation in fatty tissues and possible biomagnification through the food chain. Kawano et al. (1988) measured various components of chlordane in the fatty tissues of marine invertebrates, mammals, fish, and seabirds. Although significant levels of chlordane were measured in the tissues of tested organisms, the amount of biomagnification and extent of metabolism varied among the higher trophic level organisms. Muir et al. (1988) detected chlordane and other POPs (DDT, hexachlorobenzene, chlorobenzene) in the tissues of Arctic animals such as cod, ringed seal, and polar bears. Twelve separate components of technical chlordane were detected in the blubber of ringed seals from the Canadian Arctic, a testament to the long-term transport and persistence of POPs as a group of contaminants.

There is a body of scientific evidence supporting the concept of declining bioavailability and concomitant toxicity of environmental contaminants with increased residence time in the environment (Alexander, 1995, 2000). It is thought that as the availability of the contaminant declines due to sequestration within natural solids (Luthy et al., 1997), the risk to potential receptors declines as well (Kelsey and Alexander, 1997). However, the cited data have been gathered from a list of compounds that is not comprehensive; furthermore, the studies focused on a limited group of receptors (bacteria, worms, some simple plant studies). Consequently, there is still an unknown potential for bioaccumulation and biomagnification of POPs through certain trophic levels.

Previous work from this laboratory has shown significant amounts of chlordane in certain crops (spinach, zucchini, lettuce) grown in soil containing levels (1–18 µg/g soil) of the pesticide that had been applied 38 years ago (Mattina et al., 2000). This finding underscores unanswered questions concerning bioavailability, toxicity, and risk to human health from weathered POPs. However, the previous study (Mattina et al., 2000) focused only on the nominal or total chlordane concentration in the vegetative compartments and provided little direct information on either the movement of the contaminant in the soil or on the bioavailability of individual chlordane components. The purpose of the present study was to monitor the changes in compositional and chiral profiles of weathered chlordane through soil and contiguous vegetative compartments. In the earlier work from our laboratory, support is provided for using the sum of the three major components,  $\alpha$ -(*cis*)-chlordane;  $\gamma$ -(*trans*)-chlordane; and *trans*-nonachlor, as a reasonable approximation of weathered technical chlordane residues remaining (Mattina et al., 1999). Since two of the three major components of

chlordane (CC and TC) are chiral and possess (+) and (–) enantiomers, for the present study total chlordane concentration was operationally defined as the sum of 5 separate components: (+) and (–)  $\alpha$ -chlordane, (+) and (–)  $\gamma$ -chlordane, and *trans*-nonachlor.

Our current research on long-term weathering of chlordane from different soils has shown alterations in the compositional and chiral features of the contaminant, as compared to technical chlordane (Eitzer et al., 2001), which are dependent on sample source. Changes in the compositional features are tracked by measuring component ratios (CRs) and chiral features are tracked by measuring the enantiomer ratios (ERs, +/–). The impact of vegetation on the CRs and ERs has been uncertain. Zucchini is known to accumulate significant levels of weathered chlordane from soil. For this reason and because of the variety of its aerial tissues, zucchini was grown in chlordane-containing soil under greenhouse conditions. We report here details of the transport and fate of the individual components of this persistent contaminant through the soil and vegetation compartments. Such details are ultimately necessary to assess accurately the cycling and risk posed from specific POPs in the environment.

## 2. Experimental

### 2.1. Greenhouse trials

Five adjacent bays of dimensions 30 cm × 91 cm × 107 cm were constructed on a cement greenhouse trough. The design permitted complete separation of the soil, vegetation, and irrigation water for each bay. Bays 1, 3, and 5 were filled with soil containing weathered chlordane residues at 459 ng/g ( $\pm 30.2$ ,  $n = 7$ ) on a dry weight basis and bays 2 and 4 were filled with chlordane-free soil. To minimize the transfer of chlordane contaminated soil onto vegetation and clean soil, all five bays were covered with polyethylene and an opening was cut in the center through which the plants grew. In December 1999 the soil was fertilized and 6–8 zucchini seeds were planted in each bay. To control white fly populations, plants were sprayed with diazinon and permethrin, neither of which interferes with the analysis of chlordane. At destructive harvest, separate soil or plant samples from individual bays were composited prior to extraction and analysis (March 2000).

### 2.2. Analytical procedure

The soil extraction procedure is described in detail elsewhere (Mattina et al., 2000) and is only summarized here. For the soil, three separate fractions were collected: bulk soil was completely free of vegetation and was collected prior to planting (pre-bulk) and 90 d later

at destructive harvest (post-bulk); near-root soil fell off the roots at harvest and was contained within the expanse of the roots; the rhizosphere was the soil that remained attached to the roots. For collection of the rhizosphere soil, harvested roots were air dried for approximately 30 min and a toothbrush was used to remove the residual soil from the plant roots. Each fraction was a composite of the three bays. Each soil sample was air dried and sieved to 0.5 mm and a 3-g subsample was extracted with 50 mL of 2:3 hexane:acetone (Ultra Resi-analyzed, Fisher Scientific, Springfield, NJ) using a microwave extraction system (CEM Corporation, Mathews, NC). The extract was then concentrated, solvent exchanged to isooctane (10 mL), and passed through a glass microfiber filter (0.2  $\mu\text{m}$ , Laboratory Science Inc., Sparks, NV) for particulate removal prior to analysis.

The vegetation extraction procedure is similar to that previously employed in our research (Pylypiw, 1993; Mattina et al., 2000) with some important modifications. Root, stem, leaf, whole fruit, fruit peel, and fruit flesh tissues were extracted separately. The vegetation was rinsed thoroughly with tap water to remove residual soil or debris, with special attention paid to the roots. Ten grams of the vegetation was blended with 75 mL of 2:1 petroleum ether:2-propanol (Ultra Resi-analyzed, Fisher Scientific, Springfield, NJ); 50  $\mu\text{L}$  of 2  $\mu\text{g/mL}$  solution  $^{13}\text{C}$ -*trans*-nonachlor (Cambridge Isotope Labs, Andover, MA) was added as an internal standard. The entire mixture was then filtered through glass wool and rinsed repeatedly with distilled water. The remaining organic extract was dried over sodium sulfate. A Florisil column clean-up (US EPA, 1986) was used to remove interfering co-extractants in the vegetation; 12 cm of PR grade 60/100 mesh Florisil (magnesium silicate, US Silica, Berkeley Springs, WV) below 2 cm of anhydrous sodium sulfate (J.T. Baker, Phillipsburg, NJ) were packed into a 22-mm internal diameter fritted chromatography column, which was then pre-eluted with 50 mL petroleum ether. The extract was transferred to the column and the sodium sulfate bed over which the extract had been dried was washed with two 10-mL rinses of petroleum ether which were also transferred to the column. The column was eluted with 100 mL of petroleum ether. This fraction did not contain CC or TC, but TN was detected in the ether elution. Consequently, TN amounts in the final extract were adjusted to correct for the mass of this component lost in the ether fraction. The column was then eluted with 100 mL of 6% diethyl ether in petroleum ether and this fraction was collected in a 500-mL Kuderna–Danish flask fitted with a 10 mL concentrator tube. The extract was concentrated and solvent exchanged to a final volume of 2 mL of isooctane.

The extracts (soil and vegetation) were analyzed on a Saturn 2000 Ion Trap GC/MS (Varian, Sugar Land, TX) system fitted with a 30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$

film thickness GAMMA-DEX-120 column (Supelco, Bellefonte, PA) that permitted the separation of chiral compounds. The GC oven was programmed as follows: initial temperature 120  $^{\circ}\text{C}$  for 1 min; 20  $^{\circ}\text{C/min}$  to 155  $^{\circ}\text{C}$ ; 0.5  $^{\circ}\text{C/min}$  to 195  $^{\circ}\text{C}$ ; 20  $^{\circ}\text{C/min}$  to 220  $^{\circ}\text{C}$  for 11 min. The injection port was maintained at 230  $^{\circ}\text{C}$  and a 3  $\mu\text{L}$  splitless injection was used. After a 40 min filament delay, the mass spectrometer was turned on under the following conditions: emission current 50  $\mu\text{A}$ , target total ion current 5000 counts, maximum ionization time 25 000  $\mu\text{s}$ , multiplier offset +200 V, and scan range  $m/z$  265–430. All sample extracts were injected twice and all data are reported on the dry weight basis of the matrix.

Racemic standards of CC and TC together with achiral TN (ChemService, West Chester, PA) were used to prepare a series of calibration standards in isooctane. The individual enantiomers and *trans*-nonachlor were present at concentrations of 5, 12.5, 25, 50, 125, and 250 ng/mL; oxychlordan (OXYC), a chlordan metabolite, was present in each calibration solution at 2 $\times$  the cited amount. Although OXYC is chiral, its enantiomers were not resolved under the chromatographic conditions specified above. All standards also contained 50 ng/mL  $^{13}\text{C}$ -*trans*-nonachlor as the internal standard. A complete set of standards was injected before and after each set of sample extracts. Each sample extract was injected twice. Enantiomerically pure standards of +TC, –TC, +CC and –CC (EQ Laboratories, Atlanta, GA) were used to establish the retention order of the enantiomers.

Two ions from the most intense chlorine cluster of each component (the  $(\text{M}-\text{Cl})^+$  ion) were selected for quantitation as follows:  $m/z = 373$  and 375 for both CC and TC,  $m/z = 407$  and 409 for TN, and  $m/z = 387$  and 389 for OXYC. The extracted ion chromatograms of these ions were converted into an ASCII text file with ChemSW GC/MS file translator (Chem SW, Fairfield, CA). The files were then imported into PeakFit version 4 (SPSS Inc., Chicago, IL), using these settings for data manipulation in PeakFit: smoothing via a Fourier transform routine and integrating using the AutoFit Peaks II second derivative parameters. For each analyte the isotope ratio from each pair of ions (e.g.,  $m/z = 373$ , 375 for CC and TC) for the standards was used to establish data acceptance criteria. For the soil samples, external standard calibration curves for each analyte were generated from the sum of the two extracted ions. For the vegetation samples, calibration curves with  $^{13}\text{C}$ -TN as internal standard were generated from the sum of the two extracted ions. For each analyte the enantiomer ratios were calculated separately from each extracted ion in the pair and averaged.

### 2.3. Quality assurance

Data quality was maintained by monitoring the retention time, mass spectra, and isotope ratios of all

standards and samples. All extracts of samples passed retention time and mass spectral criteria when compared to the calibration standards. For the isotope ratios the theoretical value for ions 373:375 is 1.04 and for ions 407:409 is 0.89. For each analyte ANOVA of the isotope ratios determined for the standards across the calibration range showed no significant difference. Therefore, for each analyte the measured ratios from the calibration standards were averaged across all injections ( $n = 12$ – $16$  at each calibration level) and the standard deviation calculated. The isotope ratio of the sample extracts was measured. If the measured isotope ratio for any analyte in the sample was not within  $\pm$  two standard deviations of the average isotope ratio value from the calibration standard, the sample was excluded from the data set. By these criteria, two samples of the bulk soil and one sample of zucchini fruit flesh were excluded. All other samples met the criteria and were included in data reduction.

A certified check soil sample (Environmental Resource Associates (ERA), Arvada, CA) containing numerous pesticides including CC and TC, was extracted and analyzed by our methodology. Recovery of the two components was 113% of the certified value ( $n = 10$ ), within the acceptance limits established by the supplier.

### 3. Results and discussion

#### 3.1. Soil analysis

Pre-bulk soil was collected and analyzed prior to the start of the experiment; post-bulk, near-root, and rhizosphere soils were collected and analyzed at destructive harvest. The data from these soil analyses are summarized in Table 1. No chlordane was detected in soil samples taken from bays 2 and 4. Fig. 1A shows an extracted ion chromatogram for a representative chlor-

dane contaminated bulk soil sample. There are several observations to be made concerning these data. The total chlordane concentration in both the near-root zone and rhizosphere soils was statistically different ( $p < 0.01$ ) from the concentration in the bulk (pre- and post-) soil. The observed concentration gradient—a localized increase in chlordane levels in soil surrounding the root (near-root soil), accompanied by a localized decrease in contaminant concentration in the soil in physical contact with the root (rhizosphere)—suggests plant-assisted mobilization of the residue is occurring. The authors hypothesize that this plant-mediated mobilization is a dynamic process and that as the residue is brought into the aqueous phase from its sequestered state in the bulk soil, the contaminant may temporarily resorb as it is drawn toward the roots. Thus, localized and most likely temporary increases in the concentration of mobilized chlordane in various regions of the soil are not unexpected. White (2000) has previously observed a soil gradient for  $p,p'$ -DDE in soils under the impact of vegetation, specifically decreased concentrations of  $p,p'$ -DDE in the rhizosphere of two crops (rye and alfalfa) that accumulate significant levels of the contaminant in their roots (equivalent to the current observations for chlordane and zucchini) and localized increases in  $p,p'$ -DDE levels in the rhizosphere of a crop (beans) that did not accumulate the contaminant in its roots. White hypothesized that exudates released from the roots of the three crops served to solubilize the weathered residue, thereby facilitating its mobility and potential uptake by the plant. Hülster et al. (1994) has speculated that root exudates promote the mobilization of recalcitrant pollutants such as dioxin. Campanella and Paul (2000) described the presence of several molecules in melon root exudates and leaf tissue that increased the apparent aqueous solubility of weathered dioxin residues. Huang et al. (1998) demonstrated that certain simple organic compounds such as citric acid (structurally similar to

Table 1

Summary of total chlordane concentration (ng/g, dry weight), component ratios (TC/TN, CC/TN, CC/TC), and enantiomeric ratios (ER TC, ER CC) for soil fractions

Soil ( $n$ ) <sup>a</sup>	Chlordane (standard deviation)	TC/TN	CC/TN	CC/TC	ER (+/–)TC	ER (+/–)CC
Pre-bulk (7) <sup>b</sup>	459 A <sup>c</sup> (30.2)	1.45 A (0.052)	3.26 A (0.115)	2.25 A (0.035)	0.861 A (0.015)	1.22 A (0.014)
Post-bulk (6) <sup>d</sup>	461 A (39.2)	1.51 A (0.062)	3.43 A (0.135)	2.27 A (0.042)	0.872 A (0.030)	1.25 A (0.022)
Near-root (8)	537 B (44.1)	1.54 A (0.064)	3.42 A (0.062)	2.26 A (0.064)	0.852 A (0.015)	1.24 A (0.017)
Rhizosphere (12)	395 C (29.4)	1.51 A (0.086)	3.42 A (0.183)	2.22 A (0.048)	0.863 A (0.017)	1.23 A (0.028)

<sup>a</sup> Number of replicates.

<sup>b</sup> Bulk soil sampled prior to planting.

<sup>c</sup> Within columns, values followed by different letters are significantly different ( $p < 0.01$ ).

<sup>d</sup> Bulk soil sampled at destructive harvest.

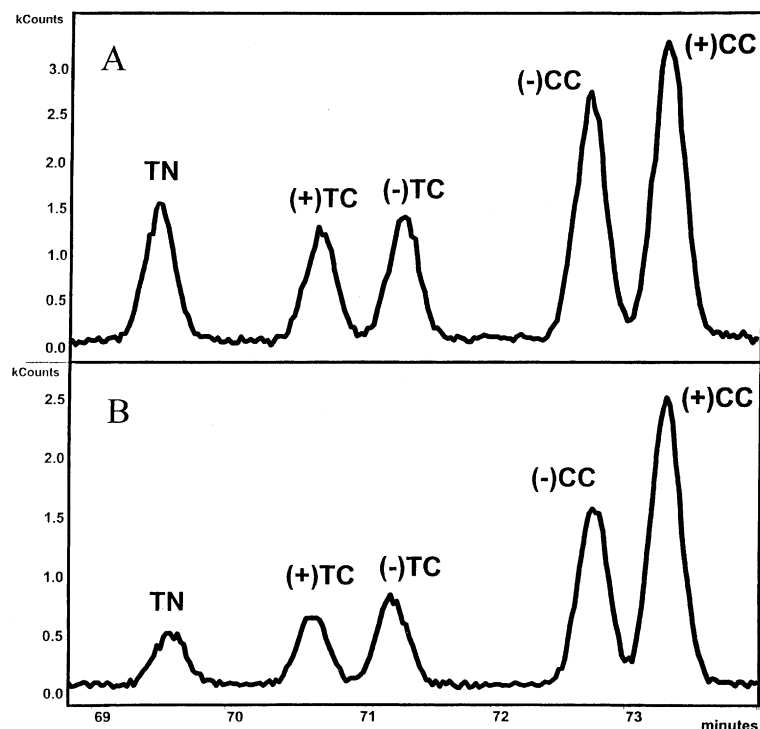


Fig. 1. Extracted ion chromatograms for bulk soil (A) and whole zucchini fruit (B). TN, *trans*-nonachlor, (+ or -)TC, enantiomers of (*trans*)-chlordane, (+ or -)CC, enantiomers of (*cis*)-chlordane.

many roots exudates) promote the desorption and plant uptake of uranium from soil. The causative factors for the observed behavior of weathered chlordane residues in soil the under the influence of vegetation remain to be determined.

As may be noted in Table 1, although total chlordane concentration changed in the various soil compartments, no significant change in the component ratios of the three constituents nor in the ERs of CC and TC in the soil during this 90 days experiment was observed. This suggests that the physical process resulting in the mobilization of the residue toward the plant acts uniformly on each of the individual chlordane components. In addition, no accumulation of OXYC was observed in the near-root or rhizosphere soils. It may also be noted from the data in Table 1 that there is a 14% reduction in the chlordane concentration in the rhizosphere relative to the bulk soil. As mentioned above White (2000) observed similar decreases in the concentration of *p,p'*-DDE in rhizosphere soils. The localized increase of mobilized chlordane in the near-root zone but not yet accessible to the plant in the rhizosphere suggests the likelihood of continued contaminant transport and uptake in successive growing seasons. The fact that any reduction in the contaminant burden of such a highly persistent and sequestered residue was achieved suggests

the possibility that phytoremediation may impact the fate and disposition of POPs in soil.

At the start of the experiment the ERs of CC and TC in bulk soil deviate from racemic, an indication that biotransformation has taken place in this soil prior to the start of the trial. A similar observation has been noted for other soils and reported in the literature (Aigner et al., 1998; Bidleman et al., 1998; Ulrich and Hites, 1998; Bidleman and Falconer, 1999; Eitzer et al., 2001). The long half-life of chlordane in soil, the observed concentration gradient in the various soil compartments under the influence of vegetation (Table 1), the absence of change in ER and in CR (Table 1) imply that non-enantioselective physical processes impact fate and transport of chlordane in soil more rapidly than enantioselective processes on the time scale of this trial.

### 3.2. Vegetation analysis

Fig. 1B shows an extracted ion chromatogram for a representative vegetation sample, whole zucchini fruit, grown in chlordane-contaminated soil. No chlordane was detected in the tissues of plants grown in bays 2 and 4. The data set for the zucchini tissues analyzed presented in Table 2 is analogous to that for the soil. Significant amounts of chlordane accumulated in the roots

Table 2

Total chlordane concentration (ng/n dry weight), component ratios (TC/TN, CC/TN, CC/TC), and enantiomeric ratios (ER TC, ER CC) in plant tissues

Tissue (n) <sup>a</sup>	Chlordane (ng/g)	TC/TN	CC/TN	CC/TC	ER (+/–)TC	ER (+/–)CC
Root (2)	5590 A <sup>b</sup> (1390)	0.671 A (0.085)	1.96 A (0.241)	2.93 A (0.021)	1.19 A (0.055)	1.18 A (0.018)
Stem (3)	3070 B (348) <sup>c</sup>	1.00 B (0.076)	3.09 B (0.251)	3.08 A (0.057)	0.87 B (0.023)	1.17 A (0.025)
Leaf (12)	484 C (149)	1.57 C (0.291)	4.84 C (0.772)	3.12 A (0.110)	0.839 B (0.024)	1.32 B (0.026)
Whole fruit (8)	299 D (79.2)	1.62 C (0.48)	4.56 B <sup>d</sup> C (1.30)	2.82 A <sup>e</sup> B (0.110)	0.771 C (0.038)	1.77 C (0.197)
Peel (5)	344 D <sup>f</sup> (90.3)	1.62 C (0.341)	4.67 C (0.611)	2.89 A (0.084)	0.797 C (0.051)	1.62 C (0.101)
Flesh (4)	187 E (42.4)	1.16 B (0.242)	3.18 B (0.660)	2.76 B <sup>g</sup> (0.090)	0.775 C (0.069)	1.83 C (0.183)

<sup>a</sup> Number of replicates.

<sup>b</sup> Within columns, values followed by different letters are significantly different at  $p < 0.05$ .

<sup>c</sup> Standard deviation.

<sup>d</sup> Different at  $p < 0.10$  from stems.

<sup>e</sup> Different at  $p < 0.10$  from roots,  $p < 0.01$  from leaves and stems.

<sup>f</sup> Significantly different at  $p < 0.10$ .

<sup>g</sup> Different at  $p < 0.05$  from peel and roots,  $p < 0.01$  from leaves and stems.

of the plant, with a substantial decline in concentration as one moves up the xylem system. This is likely due to more rapid chlordane absorption by the roots than translocation into aerial tissues. It has been known for some time that certain persistent pesticide residues such as lindane, aldrin, and heptachlor in soil can be efficiently translocated into a range of crops (Lichtenstein, 1960; Lichtenstein and Schulz, 1960; Lichtenstein et al., 1965). Chlordane is also translocated from soil into vegetation (Lichtenstein et al., 1965; Hülster et al., 1994) with zucchini and other members of the family Cucurbitaceae shown to be efficient uptakers, as well as translocators, of chlordane (Mattina et al., 2000).

The component ratios were measured in the various plant tissues and are shown in Table 2. The two component ratios in the roots (TC/TN and CC/TC) are significantly different ( $p < 0.05$ ) from values obtained in all of the soil compartments. The CC/TN in the roots is significantly different from that in near-root, post-bulk, and rhizosphere soils ( $p < 0.05$ ). Within the various tissues of the plant TC/TN and CC/TN change significantly in the order root < stem < leaf, fruit for both ratios. In other words in the aerial tissues the amount of both CC and TC increase significantly relative to the amount of TN. Changes in CC/TC are far less dramatic. At this point we can only speculate if the change in CR is due to differential transport efficiencies or variation in the kinetics of metabolism of the components.

The ERs of the two chiral components, CC and TC, were determined in the various plant tissues. Recall that the ERs of CC and TC in the rhizosphere were 1.23 and 0.86, respectively. In the roots, both ERs were significantly different from that in the soil; the ER for CC had decreased to 1.18 and the ER for TC had increased substantially to 1.19. Significant alteration in the ERs of both chiral components continued in the various aerial plant compartments, but the trends of the two compo-

nents from root to stem to leaf to fruit were in opposite directions: the ER for CC increasing and the ER for TC decreasing. It should be recalled that abiotic processes such as water solubility, volatilization, hydrolysis, and photolysis will affect both enantiomers equally since the physicochemical characteristics of the chiral components are equivalent (Buser et al., 1992; Buser and Muller, 1993). On the other hand, bacteria in natural systems routinely degrade pollutants enantioselectively. The result is a deviation of ERs from 1, the value when chlordane is introduced into the environment at application as a racemic mixture (Lichtenstein, 1960; Bidleman and Falconer, 1999). Enantioselective effects of biological processes on weathered chiral pollutants have been documented in the literature (Lichtenstein, 1960). Most likely, the resident soil microbes present in the soil employed in the greenhouse trial are responsible for enantioselective metabolism producing the observed ERs in the soil initially: 1.22 and 0.86. Within the plant very substantial and rapid changes in the ERs of the two components are noted on the time scale of this experiment. In the roots, there is an increase in the amount of –CC and +TC relative to the values in soil. However, this is followed by an enantioselective process as the analytes move through the stems, leaves, and fruit with the ERs shifting toward increased levels of +CC and –TC. The alteration in ER is not accompanied by an increase in the OXYC concentration, a primary metabolite, in the various plant tissues. Possible enantioselective processes such as preferential metabolism and enantioselective transport of the chiral components through the various plant compartments, as suggested by Jantunen and Bidleman (1998), may both play a role in plant systems.

A visualization of the trends in CR and ER through the soil and vegetation compartments is shown in Fig. 2. All the samples within a given soil or tissue compart-

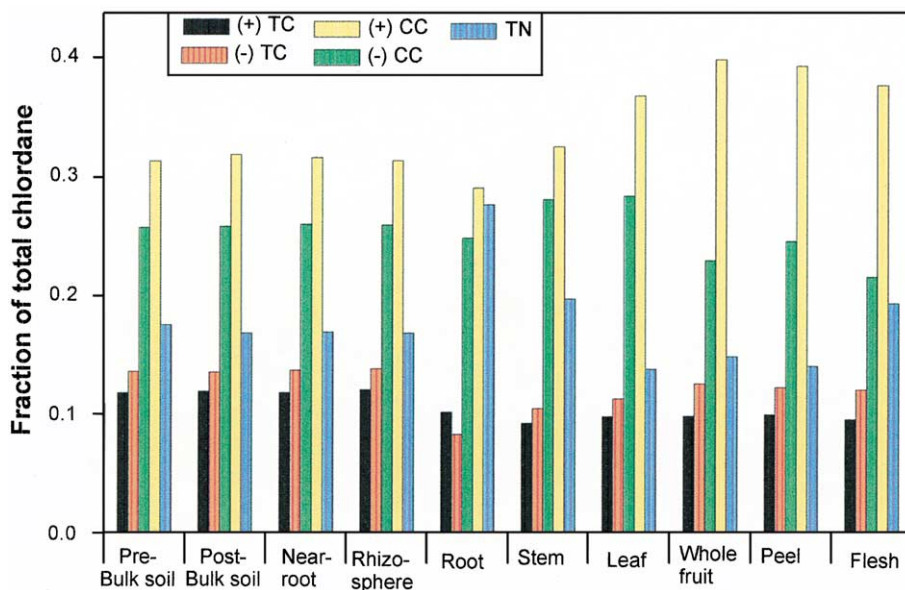


Fig. 2. Normalized component values of total chlordane for soil and plant tissues. TN, transnonachlor; (+ or -)TC, enantiomers of (*trans*-)chlordane, (+ or -)CC, enantiomers of (*cis*-)chlordane.

ment were averaged and plotted on the bar chart. The alterations in component and chiral patterns throughout the different soil and vegetative compartments are clearly apparent. The chart shows clearly the preferential increase in +CC over -CC and in -TC over +TC in moving through the aerial tissues.

Fig. 2 implies that correlations may exist between the concentration of various analytes throughout the soil and vegetation compartments. There is a significant correlation ( $r^2 = 0.82$ ) that exists between +CC and -TC as one moves through the vegetative compartments. There is also a high degree of correlation between the relative amounts of TN and CC ( $r^2 = 0.88$ ) in the plant tissues. This correlation is driven by the +CC enantiomer ( $r^2 = 0.62$ ) since there is no correlation between TN and -CC. There is also a reasonable correlation between the relative amount of TN and the relative amount of -TC ( $r^2 = 0.60$ ). Again, the processes (transport and/or metabolism) responsible for the trends indicated by the correlations of individual component concentrations remain unknown.

These data indicate that non-enantioselective translocation of certain weathered chlordane components through the soil is followed by enantioselective processes within the various tissues of the plant. The result is significant shifts of both the compositional and chiral profiles of the weathered residue. These data represent the first report in the literature that the effects of enantioselective processes in plants may be observed on a time scale equivalent to the growing cycle of the plant. Considerable work is still required to determine the ex-

act nature of the enantioselective processes. Quantitative mass balance studies are necessary to elucidate the precise nature of the component and chiral shifts observed within the various vegetative tissues. In addition, it remains to be determined if the enantioselective processes render the contaminant more or less toxic to mammalian systems. Given the current lack of knowledge concerning the cycling of persistent organic pollutants and the ultimate risk they pose to susceptible species, these findings are significant in that they indicate substantial bioavailability and biotransformation of what previously was considered to be a highly sequestered/recalcitrant contaminant.

## References

- Aigner, E.J., Leone, A.D., Falconer, R.L., 1998. Concentrations and enantiomeric ratios of organochlorine pesticides in soils from the US corn belt. *Environ. Sci. Technol.* 32, 1162–1168.
- Alexander, M., 1995. How toxic are chemicals in soil? *Environ. Sci. Technol.* 29, 2713–2717.
- Alexander, M., 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ. Sci. Technol.* 34, 4259–4265.
- Bidleman, T.F., Falconer, R.L., 1999. Enantiomer ratios apportioning two sources of chiral compounds. *Environ. Sci. Technol.* 33, 2299–2301.
- Bidleman, T.F., Jantunen, L.M., Harner, T., Wiberg, K., Wideman, J.L., Brice, K., Su, K., Falconer, R.L., Aigner, E.J., Leone, A.D., Ridal, J.J., Kerman, B., Finizio, A.,

- Alegria, H., Parkhurst, W.J., Szeto, S.Y., 1998. Chiral pesticides as tracers of air-surface exchange. *Environ. Pollut.* 102, 43–49.
- Buser, H.-R., Müller, M.D., 1993. Enantioselective determination of chlordane components, metabolites, and photoconversion products in environmental samples using chiral high-resolution gas chromatography and mass spectrometry. *Environ. Sci. Technol.* 27, 1211–1220.
- Buser, H.-R., Müller, M.D., Rappe, C., 1992. Enantioselective determination of chlordane components using chiral high-resolution gas chromatography-mass spectrometry with application to environmental samples. *Environ. Sci. Technol.* 26, 1533–1540.
- Campanella, B., Paul, R., 2000. Presence, in the rhizosphere and leaf extracts of zucchini (*Cucurbita pepo* L.) and melon (*Cucumis melo* L.), of molecules capable of increasing the apparent aqueous solubility of hydrophobic pollutants. *Int. J. Phytoremed.* 2, 145–158.
- Eitzer, B.D., Mattina, M.J.I., Iannucci-Berger, W., 2001. Compositional and chiral profiles of weathered chlordane residues in soil. *Environ. Toxicol. Chem.* 20, 2198–2204.
- Gosselin, R.E., Smith, R.P., Hodge, H.C., 1984. *Clinical Toxicology of Commercial Products*, fifth ed. Williams and Wilkins, Baltimore MD, Section III, 108–109.
- Huang, J.W., Blaylock, M.J., Kapulnik, Y., Ensley, B.D., 1998. Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyperaccumulation in plants. *Environ. Sci. Technol.* 32, 2004–2008.
- Hülster, A., Müller, J.F., Marschner, H., 1994. Soil-plant transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to vegetables of the cucumber family (Cucurbitaceae). *Environ. Sci. Technol.* 28, 1110–1115.
- Jantunen, L.M.M., Bidleman, T.F., 1998. Organochlorine pesticides and enantiomers of chiral pesticides in Arctic Ocean waters. *Arch. Environ. Contam. Toxicol.* 35, 218–228.
- Kawano, M., Tsuyoshi, I., Wada, T., Hidaka, H., Tatsukawa, R., 1988. Bioconcentration and residue patterns of chlordane compounds in marine animals: invertebrates, fish, mammals, and seabirds. *Environ. Sci. Technol.* 22, 792–797.
- Kelsey, J.W., Alexander, M., 1997. Declining bioavailability and inappropriate estimation of risk of persistent compounds. *Environ. Toxicol. Chem.* 16, 582–585.
- Lichtenstein, E.P., 1960. Insecticidal residues in various crops grown in soils treated with abnormal rates of aldrin and heptachlor. *J. Agr. Food Chem.* 8, 448–451.
- Lichtenstein, E.P., Schulz, K.R., 1960. Translocation of some chlorinated hydrocarbon insecticides into the aerial parts of pea plants. *J. Agr. Food Chem.* 8, 452–456.
- Lichtenstein, E.P., Schulz, K.R., Skretney, R.F., Stitt, P.A., 1965. Insecticidal residues in cucumbers and alfalfa grown on aldrin- or heptachlor-treated soils. *J. Econ. Entomol.* 58, 742–746.
- Luthy, R.G., Aiken, G.R., Brusseau, M.L., Cunningham, S.D., Gschwend, P.M., Pignatello, J.J., Reinhard, M., Traina, S.J., Weber Jr., W.J., Westall, J.C., 1997. Sequestration of hydrophobic organic contaminants by geosorbents. *Environ. Sci. Technol.* 31, 3341–3347.
- Mattina, M.J.I., Iannucci-Berger, W., Dykas, L., 2000. Chlordane uptake and its translocation in food crops. *J. Agr. Food Chem.* 48, 1909–1915.
- Mattina, M.J.I., Iannucci-Berger, W., Dykas, L., Pardus, J., 1999. Impact of long-term weathering, mobility, and land use on chlordane residues in soil. *Environ. Sci. Technol.* 33, 2425–2431.
- McKinney, J.J., Waller, C.L., 1994. Polychlorinated biphenyls as hormonally active structural analogues. *Environ. Health Persp.* 102, 290–297.
- Muir, D.C.G., Norstrom, R.J., Simon, M., 1988. Organochlorine contaminants in arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordane-related compounds. *Environ. Sci. Technol.* 22, 1071–1079.
- Nash, R.G., Woolson, E.A., 1967. Persistence of chlorinated hydrocarbon insecticides in soil. *Science* 157, 924–926.
- Pylypiw, H.M., 1993. Rapid gas chromatographic method for the multiresidue screening of fruits and vegetables for organochlorine and organophosphate pesticides. *J. AOAC Int.* 76, 1369–1373.
- Ulrich, E.M., Hites, R.A., 1998. Enantiomeric ratios of chlordane-related compounds in air near the Great Lakes. *Environ. Sci. Technol.* 32, 1870–1874.
- US Environmental Protection Agency, 1986. *Test Methods for Evaluating Solid Waste: Volume 1B: Laboratory Manual Physical/Chemical Methods. Method 3620A*, Washington, DC, September.
- US Environmental Protection Agency, 1990. *Pesticides and Toxic Substances*, Washington, DC, February, (EN-342), 20T-1002.
- Wania, F., Mackay, D., 1996. Tracking the distribution of persistent organic pollutants. *Environ. Sci. Technol.* 30, 390A–396A.
- White, J.C., 2000. Phytoremediation of weathered *p,p'*-DDE residues in soil. *Int. J. Phytoremed.* 2, 133–144.